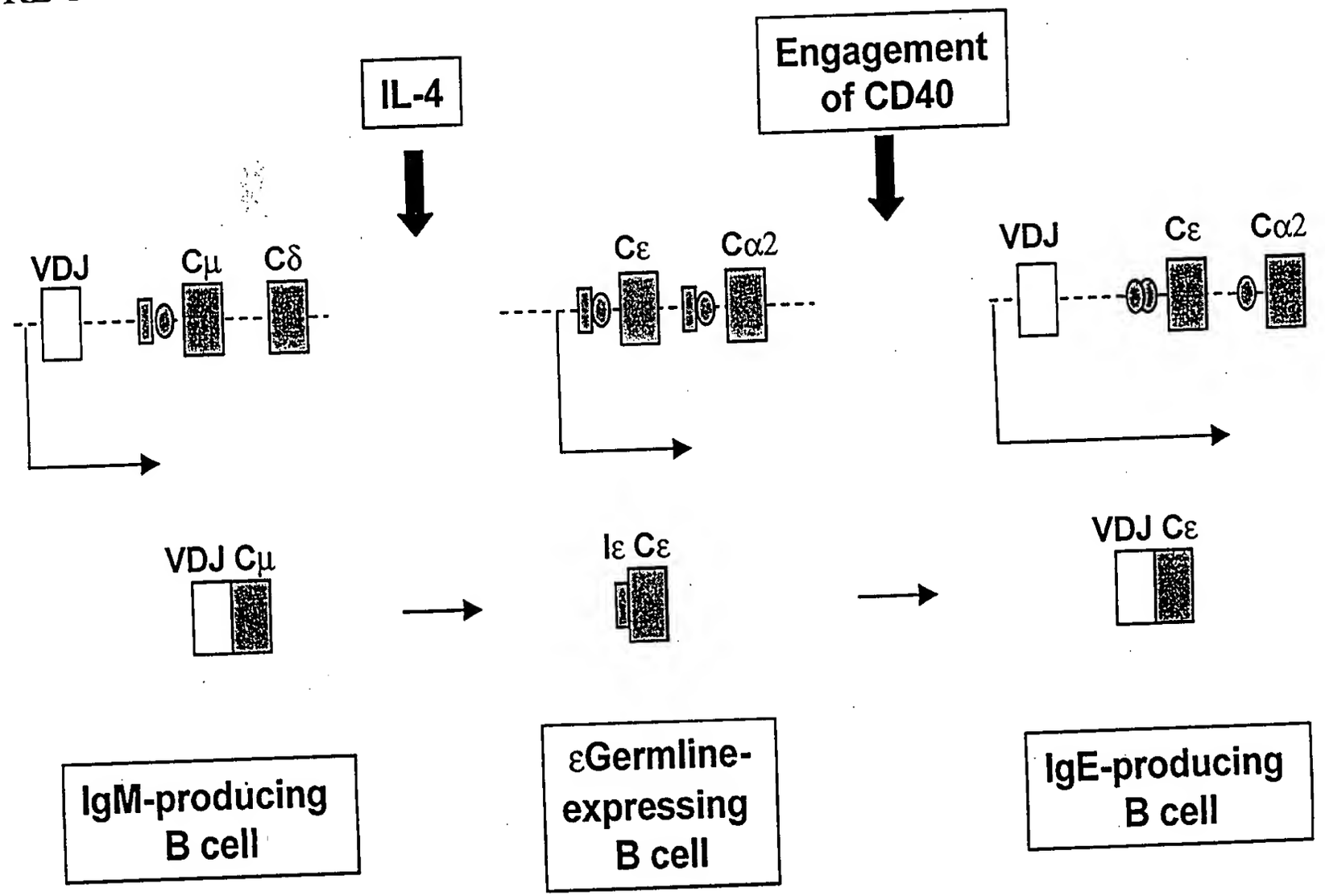
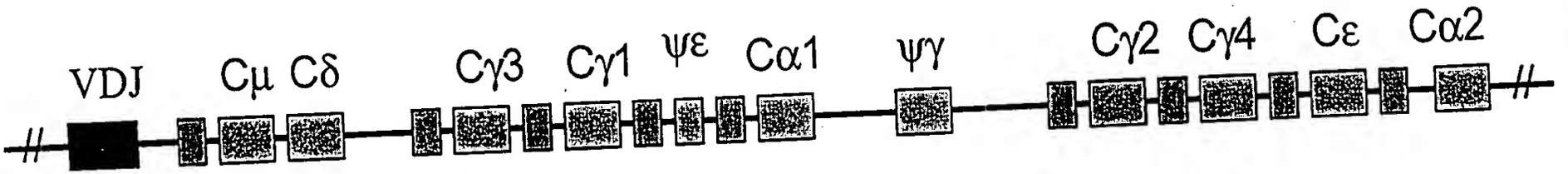
ε Germline Transcription and IgE Switching

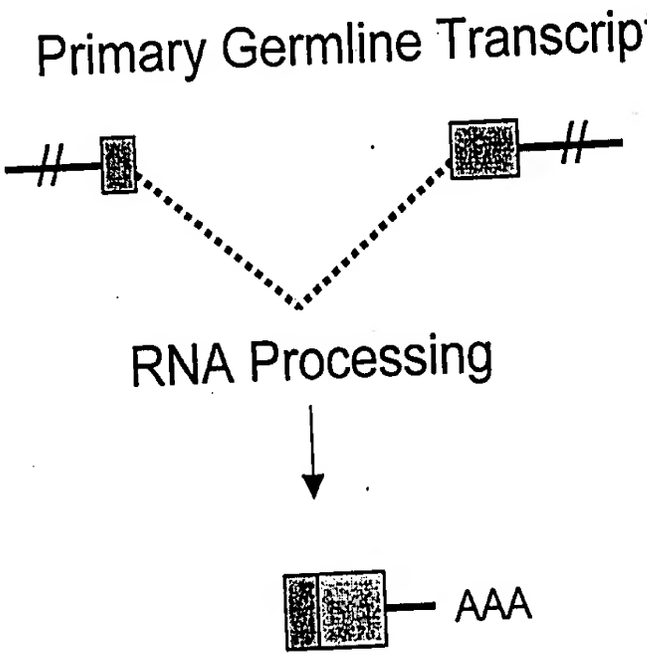
FIGURE 1



Chromosome 14 Human Heavy Chain Gene Map

FIGURE 2





= I exon

= Constant exons

FIGURE 3
1 OF 3

Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCACGTGAAGCACTACACGAATCCCA
GCCAGGATGTGACTGTGCCCTGCCCAGT
TCCCCCACCTCCCCCATGCTGCCACCCCCGACTGTCGCTGCACCGACCGGCCC

Germline Ig Epsilon Probe

GGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCC
CAAATCTTGTGACAAAACTCACACATGCCCACCG

Germline Ig Gamma 2 Pr be

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCGTG
GAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGT
AGATCACAAGCCCAGCAACACCAAGGTG
GACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCAC
CACCTGTGGCAGGACCGTCA

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGA
CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAGCTTGGGCACCCAGACCTACACCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCT
CAAAACCCCACTTGGTGACACAACTCACACATGCCCACGGTGCCCAGAGCCC
AAATCTTGTGACACACCTCCCCCGTGCC
CACGGTGCCC

FIGURE 3
3 OF 3

Germline Ig Gamma 4 Probe

GGCCAGCACCACATGGAAGCCCAAGCGGAGCCAGCACGGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGGAGAGGCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGGGCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
CTTGGGCACGAAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA
TATGGTCCCCCGTC

FIGURE 4
1 OF 3

Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-1 Probe

GGCCTGGGCCGGGCCAGGGCTCCCTCCCCACAGCAGCCCTCTTGGCAGGCAG
CCAGACGCCCGTGAGGGTGGACCTGCCA
TGAGGGCCTGCACGCCGGAGGCCGCCCACTCAGCACTGCGGGCCCTCCAGCA
GCCTGACCAGCATCCCCGACCAGCCCCA
AGGTCTTCCCGCTGAGCCTCTGCAGCACCCAGCCAGATGGGAACGTGGTCAT
CGCCTGCCTGGTCCAGGGCTTCTTCCCC
CAGGAGCCACTCAGTGTGACCTGGAGCGAAAGCGGACAGGGCGTGACCGCC
AGAAACTTCCCACCCAGCCAGGATGCCTC
CGGGGACCTGTACACCACGAGCAGCCAGCTGACCCTGCCGGCCACACAGTGC
CTAGCCGGCAAGTCCGTGACATGCCAC

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCCGGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTTGGCAGG
CAGCCAGACGCCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCCACTCAGCACTGCGGGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAACTTCCCACCTAGCCAGGATGCCTCCGGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCAC

Germline Ig Epsilon Probe

GGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCATCCACAGGCACCAAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCTTG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCCGTG

FIGURE 4
2 OF 3

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGCGCTGGGAGCACGA
GGAACATGACTGGATGCGGCAGAGCCGGC
CGTGGGGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGACCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGCAGAGCCGGCCGTGGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGG

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGCGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGATGCCAGGATGGGCATGACCGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGACCCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCTTCCACCAAGGGCCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACCTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAG

FIGURE 4
3 OF 3

Germline Ig Gamma 4 Probe

GGCCAGCACCACATGGAAGCCCAAGCGGAGCCAGCAC GGGGGAGGTGGGCA
GCCTTCAGGCACTGATGCCCACCCAGTGC
GAGACGACGGGGACCGTGGGCAGGGGCTTCCAAGCCA CAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGG CAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGGAGAC GCCCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCCGTAGG GCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAG ACCTCCGAGAGCA
CAGCCGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGG

FIGURE 5

RPA PROBES

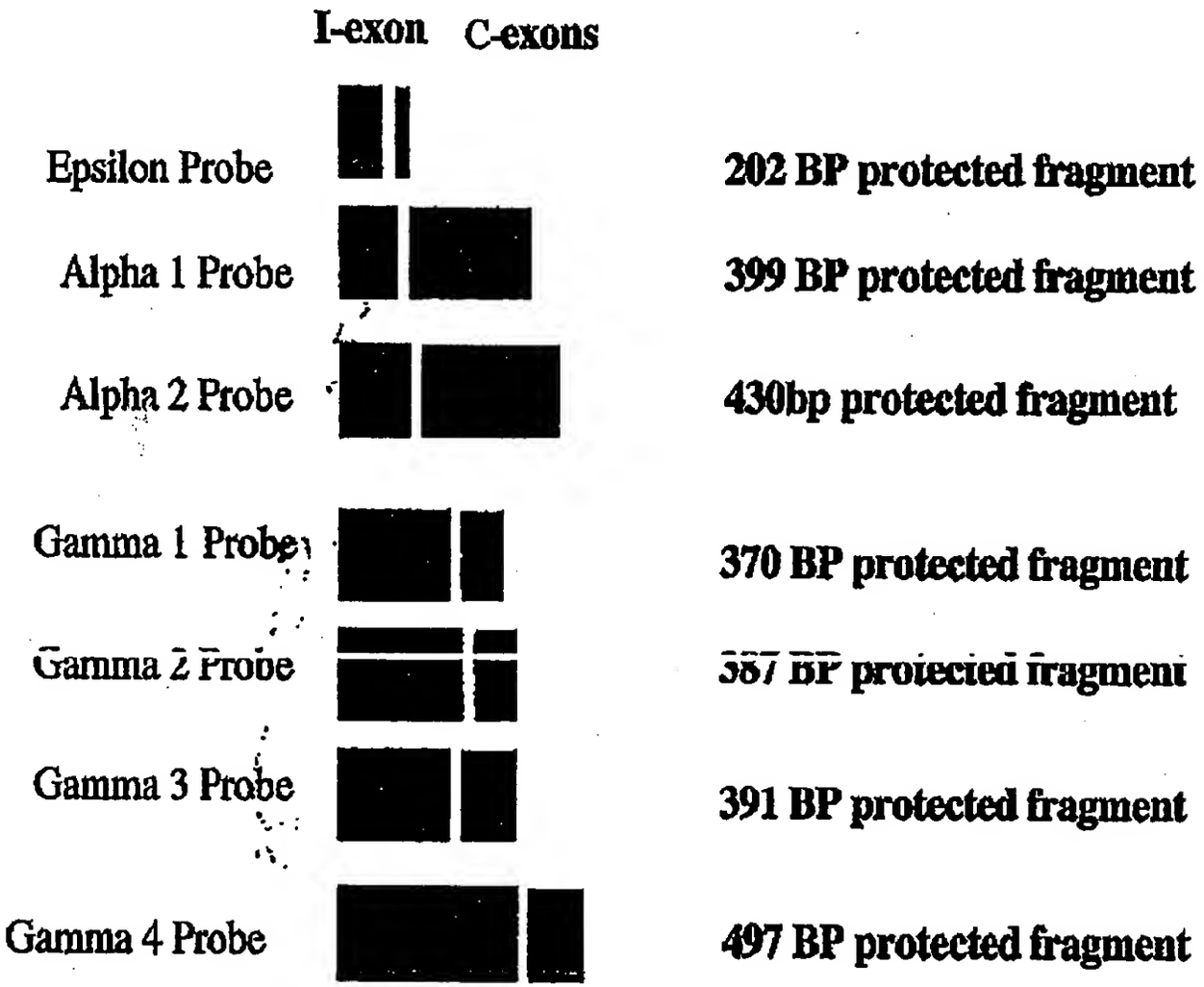
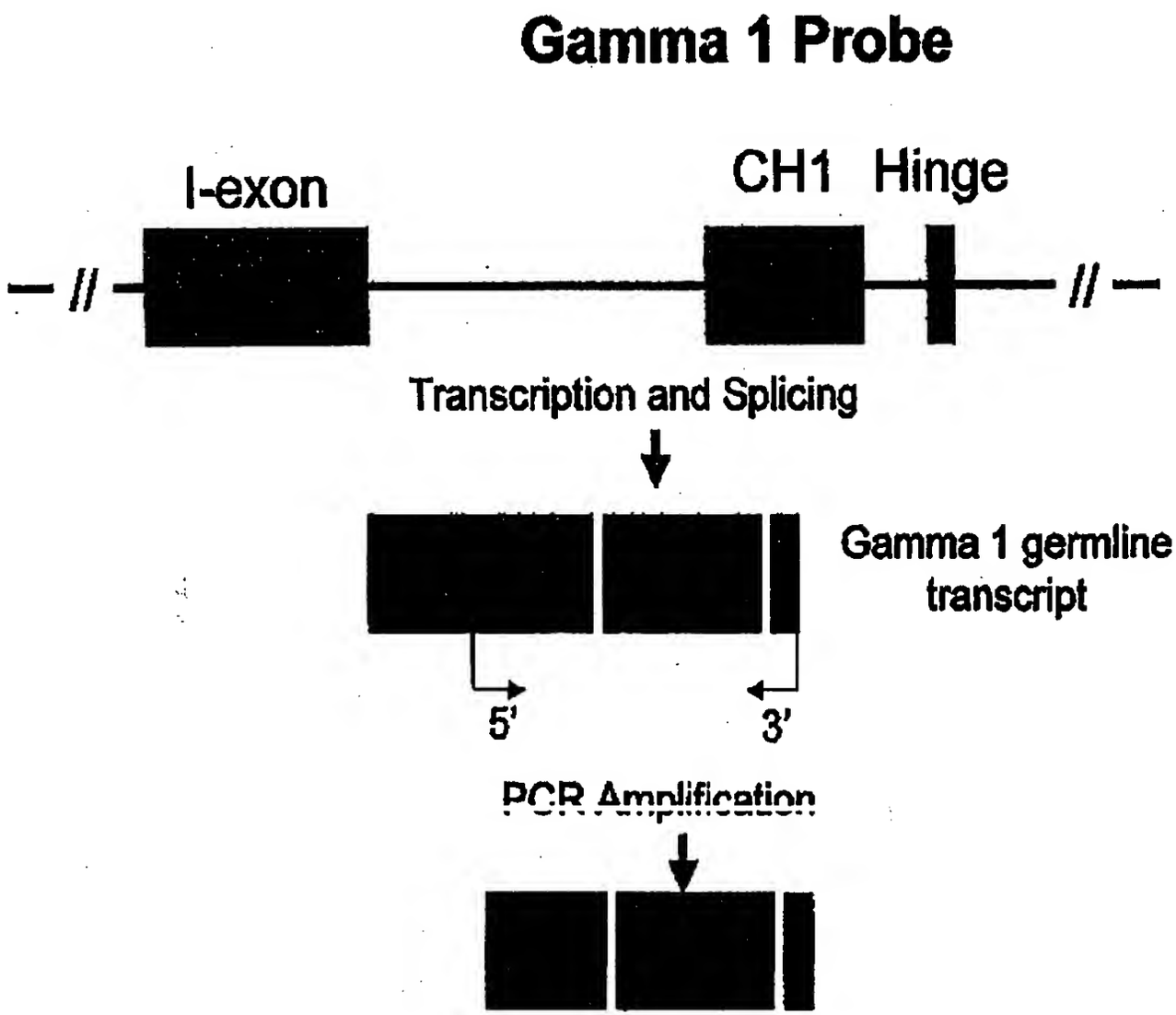


FIGURE 6



The Gamma 1 5' and 3' Primers amplified a completed probe of 370 BP

RNAse Probe Protection Assay

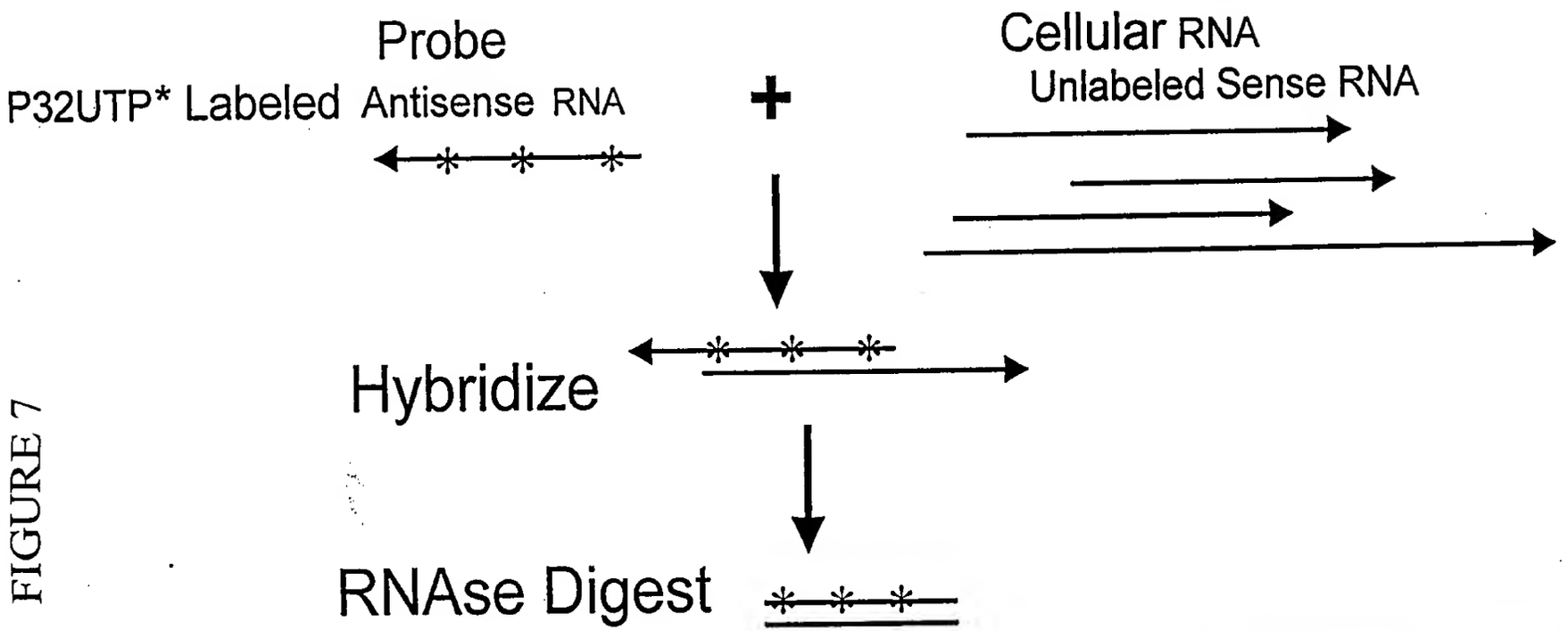
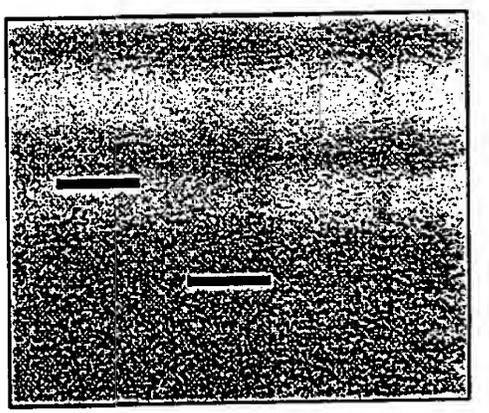


FIGURE 7

Run undigested probe vs digested protected fragment on acrylamide-Urea gel



Visualize using beta imaging equipment

FIGURE 8

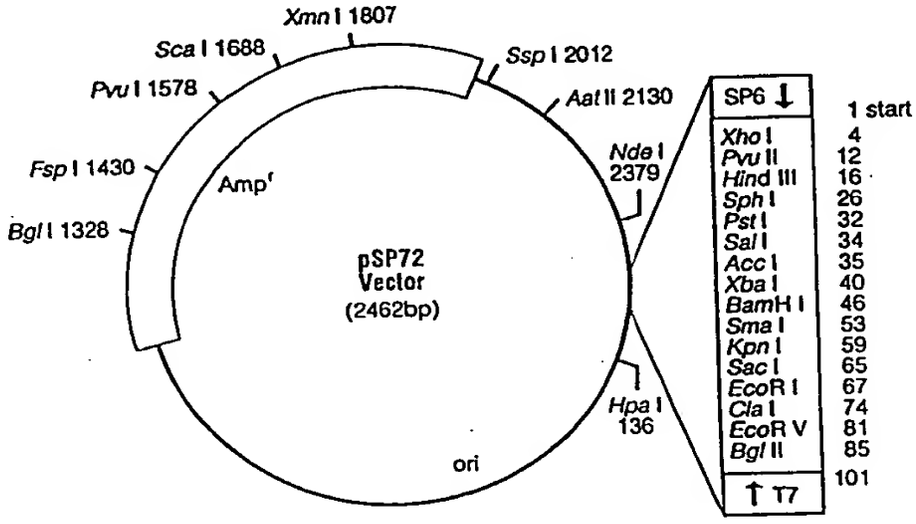


Figure 2. pSP72 Vector circle map and sequence reference points.

1. Sequence reference points:

a. SP6 RNA polymerase transcription initiation site	1
b. T7 RNA polymerase transcription initiation site	101
c. SP6 RNA polymerase promoter	2446-6
d. T7 RNA polymerase promoter	96-118
e. multiple cloning sites	4-90
f. β-lactamase (Amp^r) coding region	1135-1995

2. Specialized application:
   a. transcription *in vitro* from dual opposed promoters (For protocol information, please request Promega's Riboprobe® *in vitro* Transcription Systems Technical Manual, #TM016.)

3. The pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.

4. Blue/white screening for recombinants is not possible with the pSP72 Vector.

FIGURE 9

Accession Numbers for Germline Transcripts

Alpha - 1

L04541 = I Region Exon
BC005951 = Constant Region Exon

Alpha - 2

L04541 = I Region Exon
AL389978 = Constant Region Exon

Epsilon

X56797 = I Region Exon
J00222 = Constant Region Exon

Gamma - 1

AL122127 = I Region Exon
Z17370 = Constant Region Exon

Gamma - 2

U39934 = I Region Exon
J00230 = Constant Region Exon

Gamma - 3

AL122127 = I Region Exon
X16110 = Constant Region Exon

Gamma - 4

X56796 = I Region Exon
K01316 = Constant Region Exon